MICROPROPAGATION AND PHYTOCHEMICAL ESTIMATION OF "*SOLANUM XANTHOCARPUM"* (SCHRAD. & WENDL.)

INTRODUCTION

Solanum. L genus consists of over 2000 species distributed worldwide (Omidiji, 1983; Knapp, 1991), and is a good source of vegetable and medicinal values (Olmstead & Palmer, 1997). *Solanum xanthocarpum* one of the member of solanaceae family is having multiple medicinal values. It is a diffuse bright green perennial herb with prickly stem, leaves and calyx (Joseph *et al.*, 2012), distributed throughout India in dry places as a weed along road side and waste lands (Parmar *et al.*, 2010).

Nearly 80% of world population mainly in developing countries depends up on herbal medicines for primary health care (Parmar *et al.*, 2010) (Goldberg, 1994). Use of medicinal herbs indicates application of any plant parts such as seeds, berries, roots, leaves, bark or flowers for medicinal purposes.(Chitra *et al.*, 2011) .Reason behind the use of such herbal medicines is that they are safe, cheaper easily available and possess no side effects and considered to be the first line of defence for curing various diseases mostly in tribal and rural communities. (Dwivedi *et al.*, 2008).

Solanum xanthocarpum Schrad. & Wendl. which belongs to family solanaceae, is an important medicinal plant, commonly known as yellow berried night shade (English) (Parmar *et al.,* 2010). Other names are kantkari (Sanskrit), Bhatkatiya, Kateri (Hindi) etc. (Singh and singh, 2010). *Solanum xanthocarpum* is used for treating kasa (cough), Shwasa (bronchial asthma), jwara (fever) etc. Fruit possess anthelmentic, antipyretic, anti-inflammatory, anti-tumour activities and also used for treatment of diseases like heart malfunction & diabetes. Roots, the constituents of "Dasmul Asava" are used as expectorant, cough, asthma & chest pain. (Parmar *et al.,* 2010) (Khare, 1995). Stem, flowers and fruits employed for curing burning sensation in feet accompanied by vesicular eruptions. (Joseph *et al.*, 2012). Part of leaves are use to treat body or muscle pain & rheumatism. (Rita and Animesh, 2011) (Sharma *et al.*, 2010). Seeds are used as a diuretic. (Singh and Singh, 2010) (Singh and Hajara, 1996). (Kilankaje *et al*., 2013) reported that this plant species also posses' steroids, alkaloid, solasonine & solamargine.

It contains many alkaloids which are highly effective in many health problems such as diabetes, cholera, bronchitis, high blood pressure, etc. More attention towards this genus

should be paid to cope up with the problems like germplasm conservation . *Solanum xanthocarpum* (Schrad. & Wendl.) an herb grows as wild plant in many parts of India. Micropropagation of these plants is a good way to conserve and multiply this amazing plant species. Tissue culture is the culture and maintenance of plant cells or organs in sterile, nutritionally and environmentally supportive condition *in-vitro* (Garg *et al.*, 2011). It can also be defined as the culture of plant cells, organs or tissues in a synthetic culture medium under controlled aseptic conditions which provides the culture a suitable microenvironment for successful growth (Garg *et al.,* 2011 and Yadav *et al*., 2009). There are different types of Tissue culture which include callus culture, cells suspension culture (Bottino, 1981), organ culture (Street *et al.,* 1973), Protoplast culture etc. Various media like Murashige & skoog's (MS) media, Microbiochemical media, orchid plant culture media, White's media, Gamborg's media etc are used for *in-vitro* propagation. (Garg *et al.,* 2011).

Due to overexploitation of this medicinal herb in ayurvedic industry as it forms basis of many polyherbal preparation (Joseph *et al.*, 2012), their reserves are diminishing & facing danger of extinction (Kilankaje *et al.,* 2013). Hence, conservation of genetic diversity of medicinal plant resources is necessary, which can be accomplished by adopting alternative methods for rapid multiplication.

Therefore the objective of the present study is to generate a reliable protocol for mass propagation and conservation using tissue culture technique and estimation of its phytochemical constituents.

Review of Literature :-

Solanum xanthocarpum Schrad & Wendl. Belongs to family solanaceae an important medicinal herb in ayurvedic and mordern industry, is a common weed in India, possessing antiasthamatic, antidiabetic, antitumour, hepatoprotective & cardiovascular and various other medicinal properties. Application of such important medicinal plant by human beings, since ancient times for curing various ailments has led to the exploitation of their resources, which can be conserved by adopting an alternative method for rapid *in-vitro* multiplication through tissue culture technique. The concept of *in-vitro* culture was developed by a German physiologist, Gottlieb Haberlandt in 1902. Tissue culture involves the use of small pieces of plant tissues i.e.- tissue containing meristem in it and would develop in to an individual on culturing with suitable medium and growth condition. *In vitro* culture of important

medicinal plants have been reported by various scientists from time to time. *Curculigo orchioides* Gaertn. (Wala and Jasrai, 2003), *Entada pursaetha* DC (Vidya *et al*., 2005), *Asparagus racemosus* Wild. (Pant and joshi, 2009), *Tinospora cordifolia* (Wild.) Miers Ex Hook. F. Thoms (Khanapurkar *et al*., 2012), etc. Only few works have been reported on micropropagation of *Solanum xanthocarpum* schrad. & wendl.

Praveen *et al.,* (1982) reported formation of plantlets from mesophyll protoplasts of *Solanum xanthocarpum* using Murashige & Skoog's (MS) basal medium supplemented with different concentrations of 2,4-D (2, 4-dichlorophenoxy acetic acid), naphthalene acetic acid , kinetin and organic acids for callus induction. Rita and Animesh (2011) documented an overview of *Solanum xanthocarpum* for providing information regarding the enormous exploitation of plant species. Sundar and Jawahar (2011) reported *in-vitro* plant regeneration from leaf and stem explants of *Solanum xanthocarpum* Schrad & Wendl – an important medicinal herb. Part of work in micropropagation of *Solanum xanthocarpum* was reported by Kilankaje *et al.,* (2013) to meet the demand of good quality raw material for herbal medicine through direct *in-vitro* shoot regeneration. In *Solanum surrattense* Burm. F. a rapid micropropagation and direct organogenesis was achieved using shoot tip and leaf explants supplemented with MS (Murashige and Skoog's) basal media containing BAP (6-benzylaminopurine) and Kinetin either individually or in combination reported by Pawar *et al.,* (2002). Several investigations on micropropagations have also been reported on other members of solanaceae family. Micropropagation of *Withania sominifera* (L). Dunal. was reported by Sen and Sharma (1991) in which they reported that maximum number of shoots were obtained when MS media supplemented with 2,4-D or IBA (indole-3-butyric acid). containing very low concentrations of BAP (benzylaminopurine) at the time of initiation of shoot multiplication whereas growth regulator free MS medium found to be the best for root induction. Bhat *et al.,* (2010) reported that maximum number of shoots were obtained in *Solanum nigrum* (L.) using leaf explants inoculated in MS medium. In another research successful protocol for micropropagation of *Solanum hainanance* was developed by Loc and Kiet (2011) using shoot elongation and shoot regeneration cultures in which MS medium contained sucrose & agar supplemented with BA P which was found to be best for shoot elongation and sucrose, agar with IBA, Kinetin & BAP for shoot regeneration and also for rooting they have used MS medium containing sucrose, agar and IBA. Apart from family solanaceae, micropropagation have also been reported in some other medicinal plants. An efficient protocol for

micropropagation of *Catharanthus roseus* (L.) was generated by Mehta *et al.,* (2013) from nodal explants of stem through axillary shoot proliferation.

Medicinal plants contain a wide range of natural products called secondary metabolites or phytochemicals. Alkaloids are the largest group of phytochemicals extracted from the plants having tremendous effects on human beings. Solasodine a specific alkaloid which have been reported from most of the members of solanaceae family. Solasodine have been isolated from leaves, stem & berries of *Solanum marginatum* (Guerrero,1976), *Solanum khasianum* Clarke. (Sree *et al.,* 1982), *Solanum aspermum* Vah L.(Bhattacarya, 1984), *Solanum laciniatum*(Conner, 1987) etc. The extraction of solasodine from dry fruits & leaves of *Solanum laciniatum* Ait & the synthesis of 16-Dehydropreynenolone Acetate from solasodine by Phase- transfer catalysis were reported by Manosroj *et al.,* (2005). Parmar *et al.,* (2010) updated a review on *Solanum xanthocarpum* (Yellow Berried Night Shade) focusing a light on its phytochemical and pharmacological activities. Gogoi and Islam (2012) reported phytochemical screening of *Solanum nigrum* L. & *S. myriacanthus* Dunal from districts of upper Assam, India. Joseph *et al.,* (2012) reported therapeutic potentials of Kantakari (*Solanum xanthocarum* Schrad. & Wendl.) providing informations regarding its medicinal uses in ayurveda and activities of the drugs isolated from them.

Estimation of their phytochemical constituents for preparation of various drugs is important. And also because of immense exploitation of this plant for medicinal uses , there is a need to conserve its germplasm through micropropagation as only few works have been reported.

Objectives :-

1. Survey & collection of *Solanum xanthocarpum* from different agro climatic zones of Chhattisgarh.
2. Estimation of Phytochemical from different plant parts of *Solanum xanthocarpum.*
3. Standardization of protocol for mass multiplication through *in vitro* regeneration of *Solanum xanthocarpum.*

Noteworthy contribution in the field of proposed work :-

Plant regeneration from leaf callus of *Solanum surattense* Burm. F. was explained by Baburaj & Thamizhchelvan (1991). Ara *et al.,* (1993) reported *in-vitro* plant regeneration from leaf explant of *Solanum sisymbrifolium* Lamk in Bangladesh. Anis *et al.,* (2003) reported micropropogation of mulberry (*Morus alba* L.) through *in- vitro* culture of shoot tip and nodal explants which has close association with *Solanum* spp. in terms of growth and environmental conditions. Jawahar *et al.,* (2004) described rapid proliferation of multiple shoots in *Solanum trilobatum* L. Ramar and Nandagopalan (2011) performed rapid *in- vitro* propagation of medicinally important plant *Solanum surattense* Burm. F. Kavitha *et al.,* (2012) established direct multiple shoot regeneration from shoot tip and nodal explants of *Solanum nigrum* L. Gupta & Dutta (1938) studied chemical examination of seeds of *Solanum xanthocarpum* schrad & wendel. The Phytochemical study of *Solanum xanthocarpum* was reported by tupkari *et al.,* (1972). kusano *et al.,* (1973), they studied steroidal constituents of the plant. Dubey & Gupta (1978) isolated A new flavonol glycosides from the flowers of *Solanum xanthocarpum* schrad & Wendl. In another study of Siddiqui & faizi (1983) explained the chemical constituents of fresh berries of the plant. In a study by Singh and Singh (2010) on *Solanum xanthocarpum,* explained that the crude extract which was quantified and chemical constituent were estimated. Their study revealed presence of alkaloids, sterols, saponins, flavonoids & their glycosides and also carbohydrates, fatty acids, amino acids etc. Kavit *et al.,* (2013) performed phytochemical analysis of leaf extract of *Phyllanthus fraternus* Webster. Research made by Dixit *et al.,* (1982, 1986) stated that solasodine, an alkaloid of *S. Xanthocarpum* possesses anti-spermatogenic activity.

Proposed methodology :-

(A) Survey & collection of *Solanum xanthocarpum* from different agro climatic zones of Chhattisgarh.

1. Collection & Morphological examination of *Solanum xanthocarpum* from different agro-climatic zones of Chhattisgarh.

(B) Estimation of Phytochemical form different plant parts of *Solanum xanthocarpum.*

1. Extraction of phytochemical form different parts of plant using: Soxhlet apparatus Kumar *et al*., (2003).

The collected plant parts like (stem, leaf, flower, fruit, root) were collected and cleaned and then dried under shade.The shade dried plant material were then ground well to fine powder. After that the powdered plant material, were packed in the thimble of soxhlet apparatus and was then extracted successively with range of organic solvents like petroleum ether, chloroform, ethyl acetate, acetone , ethanol, methanol, and d.w using soxhlet extractor. The extraction was continued for 24hours. Various extracts were then filtered and kept in an oven at a particular temperature and time to evaporate the solvent from it. Greenish brown and black residues were obtained. The residue obtained were then used for preliminary phytochemical screening.

2. Qualitative estimation of phytochemicals:

a. **Preliminary phytochemical screening**.Sharma *et al.,* (2013) :Neelima *et al.,* (2011) different plant extract of *Solanum xanthocarpum* were subjected to preliminary phytochemical screening by Qualitative chemical tests which provides knowledge about various phytoconstituents such as alkaloids, flavonoids, glycosides, saponins, sterols etc. present in crude plant extract.

b. Qualitative estimation by TLC(Thin layer chromatography).Neelima *et al.,* (2011).

Thin layer chromatography technique also provides knowledge about the number of chemical constituents in the crude plant extract. TLC plates coated with layer of adsorbents like silica gel, aluminium oxide or cellulose called as stationary phase were used for the analysis. Various extracts of the plant were applied as a thin band, about 2cm from the edge, by using capillary tubes. A small amount of appropriate solvent or solvent mixture known as mobile phase was poured in to the separation chamber. The TLC plate containing the sample was placed at 45^0 angles in the development chamber covering the bottom of the plate by the solvent up to nearly 1cm.The solvent mixture was drawn up the plate through capillary action. The solvent front was marked and the plate was finally allowed to dry. The qualitative evaluation of the plate was done by determining the migrating behaviour of the separated substances given in the form of Rf value.

3. QUANTITATIVE ESTIMATION OF PHYTOCHEMICALS.

Quantitative estimation can be performed by several analytical technique like TLC Trivedi and Pundarikakshudu (2007), Spectrophotometer Saini *et al*., (2007), HPTLC(High performance thin layer chromatographic technique) Pawar *et al*., (2008): Upadhye *et al*., (2012) and various other techniques.

Quantitative analysis of HPTLC: A stock solution of standard substance (1mg/l) was prepared in suitable organic solvent. HPTLC was performed on aluminium backed HPTLC plates 10x10 cm coated with 0.2 mm layers of silica gel 60 F_{254} (E. Merck, Germany).The samples were applied on plate with band width 6 mm employing Linomat IV sample applicator (Camag, Switzerland) fitted with a microliter syringe. Linear ascending development of the plates up to distance of 80 mm was performed with mobile phase in twin-trough glass chamber. The plate was allowed to develop for 10 min and then dried at 60^{o}C for five minutes in an electric oven. The dried plates were derivatized by anisaldehyde sulphuric acid reagent in a derivatization tank and the plate was dried for 20 min at 120^{0} The well developed plate was then scanned at 546 nm in a TLC scanner (CAMAG, Switzerland). The concentration of substance in the sample was determined by explorating the percent peak area on calibration curve.

(C) Standardization of protocol for mass multiplication through *in vitro* regeneration of *Solanum xanthocarpum* using standard media. Murashige & Skoog's(MS), 1962.

EXPLANT INITIATION

1. Selection & collection of different plant parts for explant initiation.
2. Optimization of protocol for surface sterilization of explant.
3. Optimization of media for explant initiation using different concentration of cytokinins.
4. Optimization of *in-vitro* growth conditions of the explant.

SHOOT MULTIPLICATION

1. Optimization of media for shoot multiplication using different concentration & combinations of both cytokinins & auxins.
2. Optimization of *in-vitro* growth conditions of the culture under multiplication phase.
3. Extending multiplication phase up to 6-7th cycle.
4. Observations & data documentation of growth under multiplication phase.

ROOT INDUCTION

1. Optimization of media for root induction using different concentration of auxins.
2. Optimization of *in-vitro* growth conditions for root induction.
3. Observations & data documentation of growth under rooting phase.

ACCLAMITIZATION

1. Optimization of growth media in semi-controlled condition of green house for growing ex-agar plantlets.
2. Optimization of growth conditions in primary hardening stage.
3. Optimization of media for plant growth under secondary hardening stage.
4. Data documentation & observation of growth pattern in primary & secondary hardening stages.

Expected outcome of the proposed work.

- Survey and collection of plants from different agro climatic zones will provide detailed account of morphological, and agronomic traits characteristic to *Solanum xanthocarpum.*
- Micropropagation will provide rapid & mass multiplication of disease free planting material within a short span of time and plant multiplication can continue throughout the year , irrespective of season , ultimately leading to germplasm conservation.

- Phytochemical estimation will provide details of phytochemicals present in different plant parts used for preparation of various drugs, which would be helpful to check their effect on some of the serious pathogenic microbes.
- Quantitative estimation of phytochemicals present in different plant parts will provide information regarding the specific alkaloids associated to plant.

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